

WHAT IS CLAIMED IS:

- 1 1. A method of sialylating a saccharide group on a recombinant
2 glycoprotein, the method comprising contacting a saccharide group which comprises a
3 galactose or N-acetylgalactosamine acceptor moiety on a recombinant glycoprotein with a
4 sialic acid donor moiety and a recombinant sialyltransferase in a reaction mixture which
5 provides reactants required for sialyltransferase activity for a sufficient time and under
6 appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said
7 saccharide group.
- 1 2. The method of claim 1, wherein the sialic acid donor moiety is CMP-
2 sialic acid.
- 1 3. The method of claim 2, wherein the CMP-sialic acid is enzymatically
2 generated *in situ*.
- 1 4. The method of claim 1, wherein the sialyltransferase is a recombinant
2 eukaryotic sialyltransferase which substantially lacks a membrane-spanning domain.
- 1 5. The method of claim 1, wherein the sialyltransferase includes a sialyl
2 motif which has an amino acid sequence that is at least about 40% identical to a sialyl motif
3 from a sialyltransferase selected from the group consisting of ST3Gal I, ST6Gal I, and
4 ST3Gal III.
- 1 6. The method of claim 1, wherein the sialyltransferase is a recombinant
2 ST3Gal III.
- 1 7. The method of claim 6, wherein the sialyltransferase is a recombinant
2 rat ST3Gal III.
- 1 8. The method of claim 1, wherein the sialyltransferase is a recombinant
2 ST3Gal IV.

1 9. The method of claim 1, wherein the sialyltransferase is a recombinant
2 ST6Gal I.

1 10. The method of claim 1, wherein the sialyltransferase is a recombinant
2 ST3Gal I.

1 11. The method of claim 10, wherein the reaction mixture comprises a
2 second recombinant sialyltransferase, which second recombinant sialyltransferase is an
3 ST3Gal III.

1 12. The method of claim 1, wherein the sialyltransferase is a recombinant
2 bacterial sialyltransferase.

1 13. The method of claim 12, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria*
3 *meningitidis* 2,3-sialyltransferase.

1 14. The method of claim 13, wherein the bacterial sialyltransferase is a
2 *Neisseria meningitidis* 2,3-sialyltransferase.

1 15. The method of claim 12, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 16. The method of claim 15, wherein the bacterial sialyltransferase is a
2 *Photobacterium damsela* 2,6-sialyltransferase.

1 17. The method of claim 12, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Haemophilus* 2,3-sialyltransferase.

1 18. The method of claim 17, wherein the sialyltransferase is a *Haemophilus*
2 2,3-sialyltransferase.

1 19. The method of claim 12, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 20. The method of claim 19, wherein the sialyltransferase is a
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 21. The method of claim 1, wherein the sialyltransferase is produced by
2 recombinant expression of a sialyltransferase in a host cell selected from the group
3 consisting of an insect cell, a mammalian cell, and a fungal cell.

1 22. The method of claim 21, wherein the host cell is an *Aspergillus niger*
2 cell.

1 23. A method of sialylating a saccharide group on a recombinant
2 glycoprotein, the method comprising contacting a saccharide group which comprises a
3 galactose or an N-acetylgalactosamine acceptor moiety on a recombinant glycoprotein with a
4 sialic acid donor moiety and a bacterial sialyltransferase in a reaction mixture which
5 provides reactants required for sialyltransferase activity for a sufficient time and under
6 appropriate conditions to transfer sialic acid from said sialic acid donor moiety to said
7 saccharide group.

1 24. The method of claim 23, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Photobacterium damsela* 2,6-sialyltransferase.

1 25. The method of claim 24, wherein the bacterial sialyltransferase is a
2 *Photobacterium damsela* 2,6-sialyltransferase.

1 26. The method of claim 23, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria*
3 *meningitidis* 2,3-sialyltransferase.

1 27. The method of claim 26, wherein the sialyltransferase is a *Neisseria*
2 *meningitidis* 2,3-sialyltransferase.

1 28. The method of claim 23, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Campylobacter jejuni* 2,3-sialyltransferase.

1 29. The method of claim 28, wherein the sialyltransferase is a
2 *Campylobacter jejuni* 2,3-sialyltransferase.

1 30. The method of claim 23, wherein the bacterial sialyltransferase has an
2 amino acid sequence which is at least 50% identical to an amino acid sequence of a
3 *Haemophilus* 2,3-sialyltransferase.

1 31. The method of claim 30, wherein the sialyltransferase is a *Haemophilus*
2 2,3-sialyltransferase.

1 32. A method for *in vitro* sialylation of saccharide groups present on a
2 glycoprotein, said method comprising contacting said saccharide groups with a
3 sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase
4 activity for a sufficient time and under appropriate conditions to transfer sialic acid from said
5 sialic acid donor moiety to said saccharide group, wherein said sialyltransferase is present at
6 a concentration about 50 mU per mg of glycoprotein or less.

1 33. The method of claim 32, wherein the sialyltransferase is present at a
2 concentration of between about 5-25 mU per mg of glycoprotein.

1 34. The method of claim 32, wherein the sialyltransferase is present at a
2 concentration of between about 10-50 mU/ml of reaction mixture and the glycoprotein is
3 present in the reaction mixture at a concentration of at least about 2 mg/ml.

1 35. The method of claim 32, wherein the method yields a glycoprotein
2 having sialylation of at least about 80% of terminal galactose residues present on the
3 saccharide groups.

1 36. The method of claim 32, wherein the sialyltransferase is a recombinant
2 sialyltransferase.

1 37. The method of claim 36, wherein the sialyltransferase substantially
2 lacks a membrane-spanning domain.

1 38. The method of claim 32, wherein the sialyltransferase includes a sialyl
2 motif which has an amino acid sequence that is at least about 40% identical to a sialyl motif
3 from a sialyltransferase selected from the group consisting of ST3Gal I, ST6Gal I, and
4 ST3Gal III.

1 39. The method of claim 32, wherein the sialyltransferase is an ST3Gal III.

1 40. The method of claim 39, wherein the ST3Gal III is a rat ST3Gal III.

1 41. The method of claim 32, wherein the sialyltransferase is an ST3Gal IV.

1 42. The method of claim 32, wherein the sialyltransferase is an ST3Gal I.

1 43. The method of claim 42, wherein the reaction mixture comprises a
2 second recombinant sialyltransferase, which second recombinant sialyltransferase is an
3 ST3Gal III.

44. The method of claim 32, wherein the sialyltransferase is a bacterial sialyltransferase.

45. The method of claim 44, wherein the bacterial sialyltransferase is a recombinant sialyltransferase.

46. The method of claim 44, wherein the bacterial sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid sequence of a *Neisseria meningitidis* 2,3-sialyltransferase.

47. The method of claim 46, wherein the bacterial sialyltransferase is a *Neisseria meningitidis* 2,3-sialyltransferase.

48. The method of claim 44, wherein the bacterial sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid sequence of a *Photobacterium damsela* 2,6-sialyltransferase.

49. The method of claim 48, wherein the bacterial sialyltransferase is a *Photobacterium damsela* 2,6-sialyltransferase.

50. The method of claim 44, wherein the bacterial sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid sequence of a *Campylobacter jejuni* 2,3-sialyltransferase.

51. The method of claim 50, wherein the sialyltransferase is a *Campylobacter jejuni* 2,3-sialyltransferase.

52. The method of claim 44, wherein the bacterial sialyltransferase has an amino acid sequence which is at least 50% identical to an amino acid sequence of a *Haemophilus* 2,3-sialyltransferase.

1 53. The method of claim 52, wherein the sialyltransferase is a *Haemophilus*
2 2,3-sialyltransferase.

1 54. The method of claim 32, wherein the sialic acid donor moiety is CMP-
2 sialic acid.

1 55. The method of claim 54, wherein the CMP-sialic acid is enzymatically
2 generated *in situ*.

1 56. The method of claim 32, wherein the sialic acid is selected from the
2 group consisting of NeuAc and NeuGc.

1 57. A method for *in vitro* sialylation of saccharide groups present on a
2 glycoprotein, the method comprising contacting the saccharide groups with an ST3Gal III
3 sialyltransferase, a sialic acid donor moiety, and other reactants required for sialyltransferase
4 activity for a sufficient time and under conditions to transfer sialic acid from said sialic acid
5 donor moiety to said saccharide group, wherein said ST3Gal III sialyltransferase is present at
6 a concentration of less than about 50 mU per mg of glycoprotein.

1 58. The method of claim 57, wherein the method further comprises
2 contacting the saccharide groups with an ST6GalII sialyltransferase.